REMARKS

Claims 44-47, 54-56, 60-65, 72-81, and 88 - 92 are pending in the application. Claims 44, 63, and 79 are amended, claim 55 has been canceled without prejudice, and new claims 106 through 115 have been added to claim allowable subject matter. Support for the new claims is found throughout the specification. Favorable reconsideration is respectfully requested in light of the following Remarks.

II. Rejection of Claims 44-47, 54-56, 60-65, 72–81, and 88-92 under 35 U.S.C. §103(a)

The Office Action rejects Claims 44-46, 54-56, 60-65, 72 – 81, and 88-92 under 35 U.S.C. §103(a) as being unpatentable over Oprandy et al. (Journal of Clinical Microbiology, 1990, hereinafter "Oprandy"), in view of Huang et al. (U.S. Patent No. 5,712,172, hereinafter "Huang"), WHO Bulletin (Bulletin of World Health Organization, 1996, hereinafter "the WHO Bulletin") and Snowden et al. (Journal of Immunological Methods, 1991, hereinafter "Snowden"). The Office Action also rejected Claims 44-47, 54-56, 60-65, 72 – 81, and 88-92 under 35 U.S.C. §103(a) as being unpatentable over Oprandy, Huang, WHO Bulletin, and Snowden, in view of Rattanarithikuln et al. (American Journal of Tropical Medicine, 1996)(hereinafter " Rattanarithikuln") and Sithiprasasna et al. (Annals of Tropical Medicine and Parasitology)(hereinafter "Sithiprasasna"). The rejections are respectfully traversed.

It is well known that "[t]o establish prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art." *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)." M.P.E.P. § 2143.03. Accord. M.P.E.P. § 706.02(j). Moreover, the mere fact that references can be combined or modified does not render the resulting combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F2d 680, 16 U.S.P.Q 2d 1430 (Fed. Cir. 1990). To sustain an obviousness rejection, there must be a teaching or suggesting in the prior art to support the combination. It is appreciated that reasons or incentives must be provided in order to combine the cited references and it is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention. See *Ex*

parte Skinner, 2 USPQ2d 1788 (B.P.A.I. 1986) and *In re Fritch,* 23 USPQ2d 1780 (Fed. Cir. 1992).

Applicants incorporate by reference and restate the text of their response filed August 13, 2004, as though fully set forth herein. Without prejudice, in this response, independent Claims 44, 63, and 79 have been amended, and new claim 106-115 have been added. Applicants respectfully request reconsideration and allowance of the pending claims in light of the incorporated previous comments, the amendments set out herein, and the following comments.

The cited references do not teach or suggest all of the claim elements of the present invention, particularly as amended, nor do they teach or suggest combining the references to produce the claimed invention. *See MPEP §2143.*

At most, Oprandy teaches an enzyme immunoassay (EIA) for malaria antigen detection. However, the EIA is done in a "dot blot" format as opposed to a capillary flow device. There are several major differences between the EIA of Oprandy and the present invention:

1. *Capture site of Oprandy comprises immobilized antigen/specimen and is impractical as a commercial device.*

Oprandy teaches the immobilization of the antigen/analyte/specimen on the assay membrane to form the test site (p. 1701, column 2, second paragraph of "Dot immunobinding assay"; p. 1702, column 1, "Specimen processing"). This requires each assay membrane to be "spotted" or printed with the specimen to be examined to form the immobilized capture reagent at the test site. Because such a device cannot be uniformly manufactured, it is impractical as a commercial product.

In contrast, the present invention uses a capture antibody; thus, many devices having substantial uniformity can be made from a single lot of capture antibody.

2. *Unlike the present invention, the labeled antibody of Oprandy device is not specific for the target analyte.*

Oprandy teaches the use of a peroxidase-conjugated antibody specific for mouse IgG (not for the target analyte) as the labeled conjugate (p. 1701, column 2, last paragraph). Such an antibody could be used to detect any mouse IgG immunoglobulin no matter what the

target analyte. However, labeled conjugates that are not specific for the particular assay increase the chance of "background" binding.

In contrast, the present invention uses a labeled antibody conjugate specific for the target analyte. This provides an inherently "cleaner" reaction.

3. *Oprandy teaches only the use of an anionic detergent to process specimen.*

To reduce retention of the target analyte on the first membrane (premembrane), Oprandy used varying concentrations of an anionic detergent, sodium dodecyl sulfate (SDS). However, anionic detergents, such as SDS, have solubilizing and denaturing effects on protein structure; these properties are exploited in a popular method of separating proteins referred to as SDS polyacrylamide gel electrophoresis or "SDS-PAGE". Anionic detergents destroy secondary and tertiary structure of proteins necessary for specific reactions and are known to affect protein-protein interactions of the type that occur between antibodies and antigens. In fact, Oprandy indicates signal intensity was lower when the analyte protein was applied in a diluent with 0.1% SDS as compared to use of a diluent with 0.01% SDS (p. 1702, column 1, first paragraph of "Results"). To some extent, the effect of SDS on the analyte in the Oprandy system is mitigated because the specimen is added to the test membrane, dried, and then exposed to a protein-containing blocking solution without SDS for at least an hour, thereby removing some of the SDS from the analyte prior to exposure to the antibody (p. 1701, column 2, second and third paragraphs of "Dot immunobinding assay").

In the present invention, the specimen is added while still in its processing solution so, if SDS as disclosed in Oprandy were used in the processing solution, there would be no opportunity for the SDS to be washed away, and the presence of SDS in the present invention would interfere with analyte-antibody interactions, decreasing sensitivity of the assay. Further, SDS could possibly cause immobilization or release of the capture antibody leading to smearing at the test site.

In contrast to Oprandy, the present invention teaches the use of a non-ionic detergent, such as NP-40, Tween-20 or Triton X-100, as described in Example 3, for releasing antigens. Non-ionic detergents are known to disrupt cellular substructure, causing the release of proteins, without denaturing protein structure.

4. *The analyte tested in Oprandy is not from a "field-collected" arthropod.*

Three types of analyte/antigen were used in Oprandy: 1) recombinant (synthetic) malaria antigen $R32tet_{32}$; 2) isolated sporozoites; 3) laboratory-raised mosquitoes fed on cultured *P. falciparum* gametocytes and not engorged with blood. In contrast, the present invention is not limited to purified antigens or non-engorged arthropods and is compatible for use in connection with field-collected arthropods feeding on blood. This is a significant difference since Oprandy indicates that if engorged mosquitoes are used, it is necessary to eliminate the endogenous peroxidase activity of blood by treating the specimen with hydrogen peroxide (p. 1703, first paragraph of Discussion). This further teaches away from the use of the Oprandy device in connection with field-collected specimens and adds to the already lengthy processing time.

5. *Oprandy assay is labor-intensive.*

As described by Oprandy, after a field specimen is processed, the field specimen must be spotted on the membrane and dried for at least 1 hour, followed by hydrogen peroxide treatment for an undisclosed amount of time, followed by "blocking" for at least 1 hour, then exposed to the analyte-specific antibody for 1 hour, washed, placed in the enzyme-labeled antibody for 1 hour, washed, and finally placed in color development (substrate) solution for 5 minutes. Altogether, the Oprandy assay requires more than 4 hours to perform after the specimen is processed and requires more than a half-dozen manual processing steps, increasing the likelihood of error.

In the present invention, results are obtained within 10 to 30 minutes after sample processing (paragraph [0181]) and once the sample is added to the disclosed test device, no other manual processing steps are needed.

In summary, Oprandy does not teach or suggest:

a "one-step" lateral flow device for detecting arthropod-borne disease antigens;

an extraction buffer for arthropods suitable for use with a one-step lateral flow device;

use of a labeled analyte-specific antibody;

use of other labels besides peroxidase enzymes; or

detection of multiple analytes.

In contrast, the present invention has a wide application and makes use of a format that is more applicable to commercial production for use with field-collected specimens.

Indeed, one of ordinary skill in the art would not find Oprandy of relevance to the present invention, particularly in light of the significant distinctions set out above.

The deficits or Oprandy are not overcome by combination with the WHO Bulletin, nor would one of ordinary skill in the art be motivated to combine these references. The assay disclosed in the WHO Bulletin is a simple dipstick for the detection of a malaria antigen, namely Pf HRP II. That dipstick device comprises only an immobilized capture antibody at a test site and a second site with immobilized antigen serving as a positive control. Because there is no conjugate pad containing a labeled antibody or a filter for the specimen prior to contact with the labeled conjugate, the WHO assay requires multiple manual processing steps, including addition of the processed sample, addition of a detection agent (labeled antibody conjugate), and addition of a washing solution to clear hemolysis products, as illustrated in Figure 1.

Further, the WHO assay uses a lysed blood specimen so there is no disclosure regarding processing of field-collected arthropods for the release of disease-related antigens from arthropod-borne pathogens.

Thus, the WHO Bulletin does not teach or suggest:

a one-step lateral flow device for use with arthropods;

an extraction buffer for arthropods suitable for use with a lateral flow device;

use of other labels besides sulforhodamine B; or

detection of multiple analytes.

Indeed, there is no disclosure in the WHO Bulletin that suggests that while the one disclosed malaria antigen detectable in human blood samples (Pf HRP II protein) is prevalent in red blood cells of humans during a certain stage of the disease, the same protein would also be present in mosquitoes in detectable amounts.

One of ordinary skill in the art would not find the WHO Bulletin of relevance to the present invention, particularly in light of the significant distinctions set out above.

The deficits or Oprandy and the WHO Bulleting are not overcome by combination with the Snowden, nor would one of ordinary skill in the art be motivated to combine the references.

The assay described in Snowden et al. is a "plain" dipstick having only immobilized antibodies; it has no conjugate pad for containing a labeled antibody. The disclosed assay is

for the detection of species-specific immunoglobins. Snowden is distinct from the present invention in several ways:

1. *Multiple processing steps are required.*

As described under "Dipstick test method", pp. 59 – 60, the dipsticks, as prepared, have immobilized antisera and are then exposed to an analyte-containing solution for 1 hour, followed by 15 minutes of washing, then exposure to the detection agents (dye-labeled antibodies) for 1 hour, followed by brief rinsing. Figure 1 exemplifies the multiple steps required to perform the Snowden assay.

2. *No extraction buffer for disease-related arthropod-borne antigens is disclosed.*

Because the assay described in Snowden is focused on the identification of the species-source of blood, the extraction buffer disclosed is different because it need only solubilize the immunoglobulin components of the bloodmeal contained within the mosquitoes. In particular, individual mosquitoes were squashed or smeared on filter paper and dried for refrigerated storage, then, the smear was eluted in 5 ml of PBS for 1 hour. Such treatment is sufficient for rehydrating and extracting soluble serum proteins, such as immunoglobulins, but is insufficient for extracting antigens from intact organisms contained within a smeared mosquito sample. Snowden contains no description for extracting antigens from arthropod-borne organisms.

3. *Sensitivity of Snowden assay is not comparable to the present invention.*

Finally, Snowden appears to describe an alternative label to colloidal metals (p. 58, column 1), namely "disperse dyes". However, the detection limit of the assay is disclosed to be "as sensitive as 10 ng/ml" (p. 61, column 1). The detection limits of the assays disclosed in the present invention using colloidal gold as the detectable label are at least 10 times more sensitive, and in some cases, at least 100-fold more sensitive. Disperse dyes have been known in the art, yet, no commercial lateral flow product utilizes these dyes as labeling reagents.

In summary, Snowden does not teach or suggest:

a one-step lateral flow device for detecting arthropod-borne disease-related antigens;

an extraction buffer for arthropods suitable for extracting disease-related antigens;

labeled antibodies specific for disease-related antigens.

At most, Snowden contributes a concept for identifying multiple analytes; however, it does so only by using different colored labeling reagents. The present invention does not teach nor rely upon the use of multi-colored reagents to distinguish between multiple analytes, but rather, relies upon location of test sites for distinguishing target analytes. Thus, one of ordinary skill in the art would not consider Snowden relevant to the present invention, by itself or in combination with other cited references.

The deficits or Oprandy, the WHO Bulletin, and Snowden are not overcome by combination Huang, nor would one of ordinary skill in the art be motivated to combine the references.

Huang purports to teach an "improved" dipstick device wherein the "backs" of the porous materials that form various regions of an immunochromatographic assay device are laminated to a semi-rigid material and the top or front surface is partially covered with a plastic material that allows the test site and control site to be visible. The Huang device differs from the present invention in several ways:

1. *Huang requires use of a partial cover on the front of the device.*

Huang distinguishes over previous dipstick devices by requiring a plastic cover over at least a partial surface of the porous materials comprising the dipstick device. The present invention does not disclose use of a plastic cover material and is not so limited.

2. *Huang is limited to immunochromatographic devices without a casing or housing.*

At column 3, lines 60 – 62, Huang teaches the usefulness of immunochromatographic devices without plastic casings. However, the present invention is not limited to "naked" dipsticks and includes descriptions of plastic cassettes in Example 9 and Figure 3.

3. *Huang recites a separate (second) labeled antibody for binding at the control site.*

Huang limits its device to the use of a control reagent that "does not recognize or bind the analyte of question in the sample" (column 5, line 66 to column 6, line 2). Huang describes, in a preferred embodiment, the use of different colored labels, one for the analyte specific antibody and one for the control reagent. Use of a different control reagent cannot indicate, in what appears to be an otherwise negative test, whether the labeled analyte-

specific antibody was properly released from the conjugate pad and/or whether the label is still associated with (conjugated to) the analyte-specific antibody.

In contrast, the control site of the present invention is not so limited and may make use of the same labeled antibody as that capable of binding target analyte, thereby testing for release of intact analyte-specific antibody-label conjugate, although other controls can be used, as known in the art.

In summary, Huang does not teach or suggest the present invention's use of plastic covers for the front surface, a device without a casing, or a separate labeled control reagent. One of ordinary skill in the art would not consider Snowden relevant to the present invention, by itself or in combination with other cited references.

None of the above cited prior art references (nor Sithiprasasna or Rattanarithikui) reveal utilization of a sample comprising field-collected arthropods in an extraction buffer comprising a non-ionic detergent and compatible with immunoassays, in connection with a one-step immunochromatographic assay device/method for the detection of a disease-related arthropod-borne analyte, especially not for detection of multiple analytes. Extraction of protein antigens from intact organisms typically requires relatively harsh conditions because the normal structure or "association" of the biomolecules of the organism must be disrupted. However, immunoassays are based upon the ability of antigens and antibodies to interact in a "natural" manner. Thus, in making an immunoassay that is performed by simply adding an extracted arthropod specimen to the test device, the challenge is to make the extraction step and the assay/detection step compatible. This "marriage" of a liquid extracted arthropod sample used in connection with a simple one-step immunoassay has not been previously demonstrated. Further, the present invention, because of its specificity and low background when compared to some of the cited prior art, is amenable to use in detecting multiple analytes within a single device.

The Examiner asserts: i) Oprandy et al. discloses the appropriate reagents; ii) Huang, the WHO Bulletin, and Snowden teach how to make a dipstick device; and iii) that one having ordinary skill in the art would have been motivated to combine these references. Applicants respectfully assert that none of these comments is supported by the cited references.

First, Oprandy et al. does not disclose the appropriate reagents. Oprandy fails to teach an extraction buffer compatible for direct use in the disclosed dipstick assay; as discussed above, the SDS-containing extraction buffer used by Oprandy is not suitable for use with the present invention due to the denaturing effect of anionic detergents upon protein structure. None of the other cited references remedy this deficiency. Also, Oprandy does not teach or disclose the use of a labeled analyte-specific antibody and instead uses a "generic" antibody-enzyme conjugate that recognizes mouse immunoglobulin. This shortcoming is not remedied by the disclosure in the WHO Bulletin and Snowden, which fail to teach antibodies specific for arthropod-borne disease-related analytes. Further, Oprandy does not teach the use of an immobilized capture antibody specific for the analyte; instead, the specimen to be tested for the presence of a particular antigen is the immobilized reagent. This presents a major drawback for large-scale commercial production of substantially uniform test devices. Again, this deficiency is not met by the disclosures in Snowden and the WHO Bulletin, which do not teach immobilization of an antibody that recognizes an arthropod-borne disease-related analyte.

Second, Huang, WHO, and Snowden may teach how to make a dipstick device for immunoassays, but they fail to teach or disclose the dipstick device of the present invention. Neither WHO nor Snowden describe or teach a dipstick device having a conjugate pad bearing a dried or lyophilized labeled conjugate that becomes soluble when the extraction solution suspected to contain the analyte makes contact with it. While Huang teaches the use of a conjugate pad, the Huang invention is limited to an immunochromatographic device having a plastic cover on at least part of the front (top) surface of the porous materials used to make the device in keeping with the objective of providing a dipstick with substantial rigidity but without a casing. The present invention is not limited to dipsticks having a plastic cover and it also encompasses devices in cassettes or casing. Moreover, the applicants do not assert that the dipstick format of the present invention is, in and of itself, novel and non-obvious. Rather, as stated above, it is the combination of reagents and a simple "one-step" test format in connection with detection of arthropod-borne disease-related analytes that is novel and non-obvious. No combination of the references provides an adequate description of how to produce an antigen-containing extract of field-collected arthropods compatible for purposes of direct application to a one-step immunoassay device.

Even assuming for purposes of present argument, only, that the references could be combined to adequately teach the present invention, one or ordinary skill would not be motivated to combine the references to arrive at the present invention. Oprandy et al. does not contribute to the understanding of how to make an arthropod assay of the type suitable for use with the one-step immunoassay of the present invention probably because Oprandy et al. also fails to describe a one-step immunoassay. In fact, the immunoassay taught by Oprandy et al. is labor-intensive and impractical to mass-produce so it seems unlikely that one skilled in the art would look to Oprandy et al. for information. The WHO Bulletin is not even directed to detection of analytes in an extracted arthropod specimen, and it does not describe how to make a one-step immunoassay device; thus, one skilled in the art is unlikely to find it useful in developing the present invention. Indeed, although the WHO Bulletin discloses a malaria-related antigen is detectable in a lateral flow immunoassay, the antigen is only from human blood samples, requiring no extraction of antigens, and the lateral flow assay described requires multiple steps due to the simplistic design of the test device. Snowden does not describe how to extract and detect arthropod-borne antigens. Instead, Snowden describes a method to distinguish the soluble immunoglobins contained in a mosquito bloodmeal with regards to species source. Also, Snowden fails to teach a one-step immunoassay. The Examiner has suggested that Snowden teaches the identification of multiple analytes, but Snowden does so only by teaching the use of multiple different colored labels (disperse dyes). The present invention does not rely on multiple different colored labels in order to distinguish multiple analytes, so Snowden has no relevance to the present invention, and one skilled in the art would not find information useful to the development of the present invention. Huang contains no information regarding the extraction and detection of arthropod-borne antigens. Further, although Huang may disclose a one-step lateral flow device, the device is so limited in structure that one skilled in the art would be unlikely to even consider the teachings of Huang when designing a lateral flow device.

Thus, the cited references do not teach or suggest all of the claim elements of the present invention, particularly as amended, nor do they teach or suggest combining the references to produce the claimed invention. In view of the foregoing, Applicants respectfully request that the rejections be withdrawn and that the pending claims be allowed.

CONCLUSION

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Favorable consideration and prompt allowance of the application is earnestly solicited.

Should Examiner Winkler believe anything further would be desirable in order to place the application in better condition for allowance, the Examiner is invited to contact the undersigned attorney at the telephone number listed below.

It is believed that any additional fees due with respect to this paper have already been identified. However, if any additional fees are required in connection with the filing of this paper, permission is given to charge account number 18-0013 in the name of Rader, Fishman and Grauer PLLC.

Respectfully submitted,

Date: June 10, 2005

James F. Kamp, Registration No. 41,882
Rader, Fishman and Grauer PLLC
39533 Woodward Ave., Suite 140
Bloomfield Hills, Michigan 48304
(248) 594-0624
Attorneys for Applicants
Customer No.: 010291

R0297542